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<b>(21) International Application Number:</b> PCT/US93/09916 <b>(22) International Filing Date:</b> 15 October 1993 (15.10.93)  <b>(30) Priority data:</b> 07/962,955 16 October 1992 (16.10.92) US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 07/962,955 (CIP) Filed on 16 October 1992 (16.10.92)  <b>(71) Applicants (for all designated States except US):</b> SMITH-KLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). AFFINITY BIOTECH, INC. [US/US]; 305 Chelsea Parkway, Boothwyn, PA 19061 (US).		<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> CONSTANTINIDES, Panayiotis, Pericleous [US/US]; 1027 North Valley Forge Road #305, Devon, PA 19333 (US). YIV, Seang, Huoth [US/US]; 800 Rockwood Road, Wilmington, DE 19802 (US).  <b>(74) Agents:</b> DINNER, Dara, L. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).  <b>(81) Designated States:</b> JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> With international search report.
<b>(54) Title:</b> PHARMACEUTICAL EMULSION COMPOSITIONS  <b>(57) Abstract</b>  Pharmaceutical compositions in the form of microemulsions comprise an oil, a mixture of high and low HLB surfactants in which the high HLB surfactant comprises a medium-chain fatty acid salt, an aqueous phase and a biologically active agent.		

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## PHARMACEUTICAL EMULSION COMPOSITIONS

## FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions in the form of water-in-oil  
10 (w/o) self-emulsifying microemulsions, processes for their preparation and their use.

## BACKGROUND OF THE INVENTION

Microemulsions can be defined in general as thermodynamically stable,  
isotropically clear dispersions of two immiscible liquids stabilized by interfacial films of  
15 surface-active molecules. The formation of microemulsions usually involves a combination  
of three to five components, namely, an oil, water, a surfactant, a cosurfactant and an  
electrolyte. The tendency to form either a water-in-oil (w/o) or an oil-in-water (o/w)  
microemulsion is influenced by the properties of the oil and the surfactant. Surfactants are  
conveniently classified on an empirical scale known as the hydrophilic-lipophilic balance  
20 (HLB) which runs from 1 to 20 for non-ionic species and from 1 to 40 for anionic species.  
In general, (w/o) microemulsions are formed using surfactants (or emulsifiers) which have  
an HLB value in the range of about 3 to 6 while (o/w) microemulsions are formed using  
surfactants which have an HLB value in the range of about 8 to 18. It has long been  
recognized that low interfacial tension contributes to the thermodynamic stability of  
25 microemulsions. To achieve this, the surfactant should preferably exhibit low solubility in  
both the oil and water phases, and be preferentially absorbed at the water-oil interface with  
concomitant lowering of interfacial tension. When interfacial tension is less than  $2 \times 10^{-2}$   
dyn/cm, a stable microemulsion can form. General reviews of microemulsions are  
provided by Bhargava et al., Pharm. Tech., 46-53, March 1987 and Kahlweit, Science,  
30 240, 617-621, 1988.

Microemulsions are typically substantially non-opaque, that is they are transparent  
or opalescent when viewed by optical microscopic means. In the undisturbed state, they  
are optically isotropic (non-birefringent) when examined under polarized light. The  
35 dispersed phase typically comprises particles or droplets which are normally between 5 and  
200 nm in size and this gives rise to their optical transparency. These particles may be  
spherical although other structures are feasible.

The role of the co-surfactant, usually a short-chain alcohol, is to increase the interfacial fluidity by penetrating the surfactant film and consequently creating a disordered film due to the void space among surfactant molecules. The use of a co-surfactant in microemulsions is however optional and alcohol-free self-emulsifying emulsions and  
5 microemulsions have been described in the literature (see for instance, Pouton et al., *Int. Journal of Pharmaceutics*, 27, 335-348, 1985 and Osborne et al., *J. Disp. Sci. Tech.*, 9, 415-423, 1988).

There are many advantages to the use of a microemulsion over a conventional  
10 emulsion (or macroemulsion) for drug transport (delivery). Microemulsions form spontaneously, without the need for a high input of energy and are therefore easy to prepare and scale up for commercial applications; they have thermodynamic stability due to their small particle size and therefore have a long shelf life; they have an isotropically clear appearance so that they may be monitored by spectroscopic means; they have a relatively  
15 low viscosity and are therefore easy to transport and mix; they have a large interfacial area which accelerates surface reactions; they have a low interfacial tension which permits flexible and high penetrating power and, lastly, they offer the possibility of improved drug solubilization and protection against enzymatic hydrolysis. In addition, microemulsions may undergo phase inversion upon addition of an excess of the dispersed phase or in  
20 response to a temperature change and this is a property of these systems that can affect drug release from microemulsions both in vitro and in vivo. The reasons for this improved drug delivery are not however well understood.

The use of lipid-based microemulsions to enhance the bioavailability of different  
25 drugs, including peptides, has already been proposed. Thus, GB 2 222 770-A (Sandoz Ltd) describes microemulsions and corresponding microemulsion "pre-concentrates" for use with the highly hydrophobic cyclosporin peptides. Thus, a suitable pre-concentrate comprises 1,2-propylene glycol as the hydrophilic component, a caprylic-capric acid triglyceride as the lipophilic component and a mixture of a polyoxyethylene glycolated  
30 hydrogenated castor oil and glycerin monooleate (ratio 11:1) as the surfactant-cosurfactant. Such formulations may then be diluted with water, to give oil-in-water rather than water-in-oil microemulsions.

GB 2 098 865A (Sandoz Ltd) describes topical compositions in the form of  
35 microemulsions comprising a water-immiscible organic solvent, an emulsifier, a co-emulsifier, water and a (non-peptide) therapeutic agent. These formulations are said to have improved skin penetrating properties. Suitable organic solvents include mono- or

diesters of glycerol with a (C6-22) carboxylic acid, such as glyceryl caprylate (which may also act as a co-emulsifier).

5 US 4 712 239 (Muller et al.) describes multi-component systems for pharmaceutical use comprising an oil, a nonionic surfactant with an HLB value above 8 and a co-surfactant which is a partial ether or ester of a polyhydroxyl alcohol and a (C6-22) fatty alcohol or acid, which components form a "single phase" on mixing. The special properties of the system are attributed to the particular blend of surfactant and co-surfactant selected. An aqueous phase is an optional extra and the therapeutic agent may be lipophilic or  
10 hydrophilic. Such systems are said to give enhanced transdermal delivery characteristics. Amongst the examples provided, one (example 1, formulation I) has PEG (20 EO)-oleic acid glycerol partial esters (40%), caprylic-capric acid glycerol partial esters (42% monoglyceride, 24%), medium-chain triglycerides (16%) and water (20%).

15 GB 1 171 125 (Glaxo Laboratories Ltd.) describes microemulsions comprising a hydrophilic oil, a blend of low and high HLB surfactants and an aqueous phase, for use as injectable preparations. In particular, example 15 thereof contains in the lipophilic phase a mixture of coconut oil and sorbitan monooleate. The patent is concerned with improved formulations and is silent on bioavailability.

20 WO 88/00059 (Engström et al., and the corresponding paper, J. Dispersion Sci. Technol., 11, 479, 1990) discloses controlled release compositions for biologically active materials comprising an "L<sub>2</sub>-phase" and containing an unsaturated (C16-22)-fatty acyl monoglyceride and an unsaturated (C16-22)-fatty acyl triglyceride, in a ratio of from 1:1 to  
25 3:1, and a polar liquid such as water. Such an unsaturated (C16-22)-fatty acyl monoglyceride is a low HLB surfactant. There is, however, no mention of the additional inclusion of a high HLB surfactant. The existence of an L<sub>2</sub> phase had previously been described for a water/monocaprylin/-tricaprylin system by Friberg et al., J. Amer. Oil Chem. Soc., 47, 149, 1970. Again, there is no mention of the additional inclusion of a  
30 high HLB surfactant.

Physical studies have been reported on systems comprising the triglyceride trioctanoin, in combination with the medium-chain fatty acid octanoic acid and the sodium salt thereof (Friberg, S., et al., Chem. Phys. Lipids, 6, 364-372, 1971). Such systems  
35 however did not contain water or a low HLB surfactant. In addition, physical studies have also been reported on formulations comprising sodium octanoate and water in octanoic acid and which concentrate on the L-2 phase (Ekwall, P., Colloid and Polymer Sci., 266, 184-191, 279-282, 721-728, 729-733, 1150-1160, 1161-1173 and 267, 607-621, 1989).

Hayashi et al. have reported that the medium-chain fatty acid salts, sodium caprylate and sodium caprate have an enhanced effect per se on colonic drug absorption (Pharm. Res., 9, 648-53, 1992; 8, 1365-71, 1991; 6, 341-6, 1988; and 5, 786-9, 1988).

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We have now surprisingly found that further improved drug delivery characteristics may be obtained with (w/o) microemulsions by the further modification of the surfactant system.

## 10 SUMMARY OF THE INVENTION

Accordingly, the present invention provides a pharmaceutical composition comprising:

(a) an oil;

15 (b) a surfactant system comprising a mixture of high and low HLB surfactants in which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant;

(c) an aqueous hydrophilic phase; and

(d) a water-soluble biologically active agent. The pharmaceutical composition upon admixing forms a stable, self-emulsifying, water-in-oil (w/o) microemulsion.

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## DETAILED DESCRIPTION OF THE FIGURES

Figure 1 Illustrates a pseudo-ternary phase diagram of a system comprising (1) an oil and a second low HLB surfactant in a fixed ratio X, (2) an aqueous phase and (3) a free fatty acid and fatty acid salt in a fixed ratio Y.

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Figure 2 Illustrates a pseudo-ternary phase diagram of the system comprising CAPTEX 8000 and CAPMUL Cg (ratio 2:1), caprylic acid and sodium caprylate (ratio 3:1) and saline.

Figure 3 Illustrates a pseudo-ternary phase diagram of the system comprising CAPTEX 8000 and CAPMUL Cg (ratio 2:1), caprylic acid and sodium caprylate (ratio 3:1) and de-ionized water.

30

Figure 4 Illustrates a pseudo-ternary phase diagram of the system comprising CAPTEX 355 and CAPMUL MCM (ratio 3:1), caprylic acid and sodium caprylate (ratio 3:1) and saline.

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Figure 5 Illustrates a pseudo-ternary phase diagram of the system comprising CAPTEX 355 and CAPMUL MCM (ratio 3:1), capric acid and sodium caprate (ratio 3:1) and saline.

Figure 6 Illustrates a pseudo-ternary phase diagram of the system comprising CAPTEX 355 and CAPMUL MCM (ratio 3:1), caprylic acid and sodium caprate (ratio 3:1) and saline.

Figure 7 Illustrates a pseudo-ternary phase diagram of the system comprising CAPTEX 200, IMWITOR 308 (ratio 4.5:1), caprylic acid and sodium caprate (ratio 2.5:1) and saline.

#### DETAILED DESCRIPTION OF THE INVENTION

As noted above the present invention comprises a pharmaceutical composition which has

- (a) an oil;
- (b) a surfactant system comprising a mixture of high and low HLB surfactants in which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant;
- (c) an aqueous hydrophilic phase; and
- (d) a water-soluble biologically active agent;

which on admixing form stable, self-emulsifying, water-in-oil (w/o) microemulsions.

Earlier work in this area disclosed that useful (w/o) microemulsions may be prepared having a lipophilic phase which is either a mixture of a medium-chain fatty acyl triglyceride oil and a low HLB surfactant which is a medium-chain fatty acyl mono- or diglyceride or a mixture thereof (Constantinides, P., WO93/02664, published 18 February 1993) or a mixture of a long-chain fatty acyl triglyceride oil and a low HLB surfactant which is a long-chain fatty acyl mono- or di-glyceride or a mixture thereof or a sorbitan long-chain fatty acyl ester (Constantinides, P., WO93/02665, published 18 February 1993). The microemulsions also included a high HLB surfactant which is a conventional non-ionic surfactant such as TWEEN 80.

The inclusion in a microemulsion of a medium-chain fatty acid salt has unexpectedly been found to further enhance the absorption of a biologically active agent when administered in formulations according to the invention.

It will be appreciated by the skilled man that the oil and the low HLB surfactant will together form a continuous lipophilic phase.

The term "medium-chain", as used herein, refers to a fatty acyl chain having from 6 to 12, preferably 8 to 10 carbon atoms which may be branched or unbranched, preferably unbranched and which may be optionally substituted.

The term "long-chain", as used herein, refers to a fatty acyl chain which may be saturated, mono-unsaturated or poly-unsaturated, having from 14 to 22, preferably 16 to 18 carbon atoms which may be branched or unbranched, preferably unbranched, and which may be optionally substituted.

Suitable oils for use in the lipophilic phase are those oils which are pharmaceutically acceptable and include fatty acyl triglycerides (fatty acyl triesters of glycerol), fatty acyl diesters of propylene glycol and mixtures thereof. The fatty acyl moieties may be of medium-chain length or of long-chain length fatty acyl moieties or may be mixtures thereof.

Suitable fatty acyl triglycerides and fatty acyl diesters of propylene glycol for use in the present invention may be of natural, semi-synthetic or synthetic origin and may include blends of different fatty acyl triglycerides and/or fatty acyl diesters of propylene glycol. Such blends include not only physical blends of medium- and long-chain fatty acyl triglycerides and/or diesters but also triglycerides and/or diesters which have been chemically modified, by for instance, interesterification, to include a mixture of medium- and long-chain fatty acyl moieties. Suitable such triglycerides and diesters are readily available from commercial suppliers.

In preferred medium-chain fatty acyl triglycerides, the fatty acid composition comprises caprylic (C<sub>8</sub>) acid optionally admixed with capric (C<sub>10</sub>) acid, for instance from 50 to 100% (w/w) of caprylic acid and from 0 to 50% (w/w) of capric acid triglycerides. Suitable examples include those available under the trade names MYRITOL; CAPTEX (Karlshams Lipid Specialties, Columbus OH), for instance CAPTEX 300, CAPTEX 350, CAPTEX 355, CAPTEX 850 and CAPTEX 8000; MIGLYOL (BASF), for instance the grades MIGLYOL 810, MIGLYOL 812 and MIGLYOL 818 (which also comprises a linoleic acid triglyceride) and MAZOL 1400 (Mazer Chemical, Gurnee, IL.). The fatty acid content of representative products is given in the following table (manufacturer's data):

Product	Caprylic %	Capric %	Other %
CAPTEX 355	55	42	C6, 2
CAPTEX 8000	98.5	<1	<1
MIGLYOL 810	65-75	25-35	C6, 2
MIGLYOL 812	50-60	30-45	C6, 2, C12, 5



Suitable long-chain fatty acid triglycerides may be conveniently obtained from neutral plant, vegetable and fish oils such as shark oil, coconut oil, palm oil, olive oil, sesame oil, peanut oil, castor oil, safflower oil, sunflower oil and soybean oil, which oils  
5 may be in their natural state or partially or fully hydrogenated. Soybean oil consists of oleic acid (25%), linoleic acid (54%), linolenic acid (6%), palmitic acid (11%) and stearic acid (4%) triglycerides while safflower oil consists of oleic acid (13%), linoleic acid (76%), stearic acid (4%) and palmitic acid (5%) triglycerides. Suitably in such long-chain fatty acid triglycerides, the major fatty acid components are C<sub>18</sub>-saturated,  
10 monounsaturated or polyunsaturated fatty acids, preferably C<sub>18</sub>-monounsaturated or polyunsaturated fatty acids, such as oleic, linoleic and linolenic acid.

Other suitable triglycerides include interesterified triglycerides which may be derived synthetically by chemically reacting blends of medium- and long-chain  
15 triglycerides, for instance triglycerides containing caprylic and capric acid moieties and vegetable oils rich in oleic or linoleic acids. Examples of suitable such interesterified triglycerides include the products available from Karlshams Lipid Specialties, as CAPTEX 810A - D and 910A - D, which typically contain from 30 to 80% capric and caprylic acids, 10 to 50% linoleic acid (810 series) or 10 to 60% oleic acid plus up to 5% linoleic acid (910  
20 series), and up to 25% of other acids.

Suitable fatty acyl diesters of propylene glycol include medium- and long-chain fatty acyl diesters. Preferably the diester is formed from medium-chain fatty acids, more preferably from caprylic and capric acids, most preferably from caprylic acid. Preferred  
25 diesters comprise from about 50 to 100% caprylic acid and from 0 to 50% capric acid. A suitable thereof is the product CAPTEX 200 (Karlshams Lipid Specialties) which comprises caprylic acid (68%), capric acid (27%) and caproic acid (4%) (manufacturer's data).

30 Suitable medium-chain fatty acid salts will be pharmaceutically acceptable water-soluble salts, for instance alkali metal salts, such as sodium and potassium salts, or ammonium or quaternary ammonium salts. Preferably, the salt is a salt of caprylic or capric acid, of which the salts sodium caprylate and sodium caprate are preferred. Sodium caprylate and sodium caprate have estimated HLB values of 23 and 21 respectively.  
35 Suitably the salt form has an HLB value in the range of 20 to 25.

Suitable non-ionic high HLB surfactants include:

(a) polyoxyethylene fatty acyl esters, for example polyoxyethylene stearic acid esters of the type available under the trade name MYRJ (ICI Americas, Inc.), for instance the product MYRJ 52 (a polyoxyethylene 40 stearate);

5 (b) polyoxyethylene-sorbitan fatty acid esters (polysorbates), for example the mono- and tri-lauryl, palmityl, stearyl and oleyl esters, for instance the polyoxyethylene sorbitan monooleates available under the trade name of TWEEN (ICI Americas Inc.), such as TWEEN 20, 21, 40, 60, 61, 65, 80, 81 and 85, of which class TWEEN 80 is especially preferred;

10 (c) polyoxyethylene glycol long-chain alkyl ethers, such as polyoxyethylated glycol lauryl ether; and

(d) polyoxyethylene glycol long-chain alkyl esters, such as PEG-monostearate.

For use herein, the non-ionic high HLB surfactant preferably has an HLB value in the range of 13 to 20.

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In microemulsions of the present invention, a non-ionic high HLB surfactant may be usefully included as an auxiliary high HLB surfactant to produce microemulsions which can solubilize a larger amount of aqueous phase. Such microemulsions are, however, generally comparatively more viscous than those in which the non-ionic high HLB  
20 surfactant is absent. Suitably, the ratio of the fatty acid salt to the non-ionic high HLB surfactant is at least 1:1.

Suitable low HLB surfactants for use in the present invention include fatty acyl monoglycerides, fatty acyl diglycerides, sorbitan long-chain fatty acyl esters and medium-  
25 chain free fatty acids, as well as mixtures thereof. Suitable mono- and di-glycerides may each include blends of different fatty acyl mono- and di-glycerides and the fatty acyl moieties may be medium- or long-chain or a mixture thereof.

Suitable medium chain fatty acyl mono- and di-glycerides are formed from caprylic  
30 and capric acids. Suitable blends comprise from about 50 to 100% caprylic acid and from 0 to 50% capric acid. Mixtures of mono- and di-glycerides preferably comprise at least 50, more preferably at least 70% by weight of monoglycerides. Suitable commercial sources of these include the products available under the trade name CAPMUL (Karlsham Lipid Specialties), for instance the products CAPMUL MCM which comprises monoglycerides  
35 (77%), diglycerides (21%) and free glycerol (1.6%), with a fatty acid composition which comprises caproic acid (3%), caprylic acid (67%) and capric acid (30%) and CAPMUL Cg which has monoglycerides (70 - 90%), diglycerides (10 - 30%) and free glycerol (2 - 4%), with a fatty acid composition which comprises at least 98% caprylic acid (manufacturer's

data for Capmul products is expressed as oleates; actual C8/10 mono- and diglyceride content of about 45%, respectively).

In a preferred embodiment of the present invention, the low HLB surfactant contains a mixture of mono- and diglycerides having at least about 80% by weight, preferably at least about 90% by weight, and more preferably at least about 95% by weight of a caproic, caprylic, capric monoglyceride or mixtures thereof, preferably a caproic, caprylic, capric monoglyceride or mixtures thereof, more preferably a caprylic, capric monoglyceride or mixtures thereof. Commercial examples of these surfactants include Imwitor 308 (Huls America, Inc.) which has about 80-90% wt. caprylic monoglycerides; and Glycerol Monocaprylin, manufactured as 1-monooctanoyl-rac-glycerol (Sigma Chemicals) having about 99% wt. caprylic monoglycerides; and Glycerol Monocaprate, manufactured as 1-monodecanoyl-rac-glycerol (Sigma Chemicals) having about 99% wt. capric monoglycerides.

Suitable long-chain fatty acyl monoglycerides include glycerol monooleate, glycerol monopalmitate and glycerol monostearate. Suitable commercially available examples of such include the products available under the trade names MYVEROL, such as MYVEROL 18-92 (a sunflower oil monoglyceride) and 18-99 (a rapeseed oil monoglyceride), MYVATEX and MYVAPLEX, respectively, from Eastman Kodak Chemicals, Rochester, New York. A further useful long-chain fatty acyl monoglyceride-containing product is ARLACEL 186 (available from ICI Americas Inc.) which includes, in addition to glycerol monooleate, propylene glycol (10%). The main fatty acids of MYVEROL 18-92 are oleic acid (19%), linoleic acid (68%) and palmitic acid (7%) while those of MYVEROL 18-99 are oleic acid (61%), linoleic acid (21%), linolenic acid (9%) and palmitic acid (4%). Suitably in such long-chain monoglycerides, the major fatty acid component is a C18-saturated, monounsaturated or polyunsaturated fatty acid, preferably a C18-monounsaturated or polyunsaturated fatty acid. In addition, diacetylated and disuccinylated versions of the monoglycerides such as the product available under the trade name MYVATEX SMG are also useful.

Suitable sorbitan long-chain fatty acyl esters for use in the present invention include sorbitan monooleate, available commercially under the trade names SPAN 80 and ARLACEL 80 and sorbitan sesquioleate, available commercially under the trade names SPAN 83 and ARLACEL 83.

Suitable medium-chain fatty acids for use in the present invention include caprylic and capric acids and mixtures thereof.

Preferably, microemulsions of the present invention comprise a medium-chain fatty acid salt and a medium-chain fatty acid which is preferably the corresponding free fatty acid, as herein before defined. Suitable combinations include sodium caprylate/caprylic acid and/or sodium caprate/capric acid. Suitably, the ratio of free fatty acid to fatty acid salt is in the range of from about 10:1 to 1:1, more suitably from about 4:1 to 1:1. Preferably, the surfactant system also comprises, in addition to the free fatty acid, a further (second) low HLB surfactant, such as a fatty acyl monoglyceride, fatty acyl diglyceride, mixture of mono- and di-glycerides or a sorbitan long-chain fatty acyl ester, preferably a fatty acyl monoglyceride, as hereinbefore defined.

10

Suitably the low HLB surfactant will have an HLB value in the range of about 2.5 to 8. The HLB values of the products CAPMUL MCM, MYVEROL 18-99, ARLACEL 80, ARLACEL 83 and ARLACEL 186 are respectively about 5.5 to 6, 3.7, 4.3, 3.7 and 2.8 while the HLB of caprylic and capric acids are 5.8 and 4.8 respectively. The estimated HLB of 1-monocaprylin is about 8.0.

15

The blend of the high and low HLB surfactants will preferably have an HLB value in the range of from about 5 to 14, preferably 7 to 13.

20

In a preferred embodiment of the present invention, microemulsions comprise medium-chain fatty acyl components, such as those derived from caprylic and capric acids, especially those derived from caprylic acid. Accordingly, preferred microemulsions include blends of CAPTEX 355, 810, CAPTEX 8000 or CAPTEX 200, particularly CAPTEX 8000; CAPMUL MCM or CAPMUL Cg, particularly CAPMUL Cg; and caprylic acid/sodium caprylate and/or capric acid/sodium caprate, particularly caprylic acid/sodium caprylate.

25

As used herein, the term "biologically active material" refers not only compounds which have use as therapeutic and/or prophylactic agents (hereinafter referred to as "drugs") but also compounds which may be of use as diagnostic agents. Such materials will be soluble in the hydrophilic phase and have an HLB value of at least that of the high HLB surfactant(s) used in the formulation, to ensure that the drug is preferentially dissolved in the hydrophilic rather than the lipophilic phase. Such materials include both peptides and non-peptides. Suitable peptides include not only small peptides but also larger peptides/polypeptides and proteins. Suitable such peptides preferably have a molecular weight from about 100 to 10,000, more preferably from about 100 to about 6,000. Especially preferred are peptides having from 2 to 35 amino acid moieties. Higher

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molecular weight peptides, even those with a molecular weight of above 10,000, up to about 50,000, may also be accommodated in microemulsions of the present invention.

Suitable small peptides have from about 2 to about 10, more preferably from about 2 to about 6 amino acid moieties. Preferred small peptides include the fibrinogen receptor antagonists (RGD containing peptides) which are tetrapeptides with an average molecular weight of about 600. These peptide antagonists are highly potent platelet aggregation inhibitors at plasma levels as low as 1 pmol/ml. Preferred fibrinogen antagonists include the peptide cyclo(S,S)-Na-acetyl-Cys-(Na-methyl)Arg-Gly-Asp-Pen-NH<sub>2</sub> (Ali et al., EP 0 341 915, whose disclosure is herein incorporated by reference in its entirety) and the peptide cyclo(S,S)-(2-mercapto)benzoyl-(Na-methyl)Arg-Gly-Asp-(2-mercapto)-phenylamide (EP 0 423 212, whose disclosure is herein incorporated by reference in its entirety). Other fibrinogen antagonists useful in the present invention are those peptides disclosed by Pierschbacher et al., WO 89/05150 (US/88/04403); Marguerie, EP 0 275 748; Adams et al., U.S. 4,857,508; Zimmerman et al., U.S. 4,683,291; Nutt et al., EP 0 410 537, EP 0 410 539, EP 0 410 540, EP 0 410 541, EP 0 410 767, EP 0 410 833, EP 0 422 937 and EP 0 422 938; Ali et al., EP 0 372 486; Ohba et al., WO 90/02751 (PCT/JP89/00926); Klein et al., U.S. 4,952,562; Scarborough et al., WO 90/15620 (PCT/US90/03417); Ali et al., PCT/US90/06514 and PCT/US92/00999; the peptide-like compounds disclosed by Ali et al., EP 0 381 033 and EP 0 384 362; and the RGD peptide cyclo-Na-acetyl-Cys-Asn-Dtc-Amf-Gly-Asp-Cys-OH (in which Dtc is 4,4'-dimethylthiazolidine-5-carboxylic acid and Amf is 4-aminomethylphenylalanine).

The RGD peptide may be usefully included in the microemulsion formulation in an amount up to about 400mg/g of the hydrophilic phase or from 0.1 to 40 mg/g of the formulation.

Other peptides useful in the present invention include, but are not limited to, other RGD containing peptides such as those disclosed by Momany, US 4,411,890 and US 4,410,513; Bowers et al., US 4,880,778, US 4,880,777, US 4,839,344; and WO 89/10933 (PCT/US 89/01829); the peptide Ala-His-D-Nal-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> (in which Nal represents  $\beta$ -naphthylalanine) and the peptides disclosed by Momany, US 4,228,158, US 4,228,157, US 4,228,156, US 4,228,155, US 4,226,857, US 4,224,316, US 4,223,021, US 4,223,020, US 4,223,019 and US 4,410,512.

Other suitable peptides include hexapeptides such as the growth hormone releasing peptide (GHRP) His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>, (Momany, US 4,411,890) and related analogs or homologs thereof, such as but not limited to, His-D-Phe-Ala-D-Phe-Lys-

Gln-Gly-NH<sub>2</sub>, Hong et al., USSN 07/951500 the disclosure of which are herein incorporated by reference in their entirety). This may usefully be included in an amount up to about 250mg/g of the hydrophilic phase or from 0.1 to 25mg/g of the formulation.

5           Suitable larger polypeptides and proteins for use in microemulsions of the present invention include insulin, calcitonin, elcatonin, calcitonin-gene related peptide somatostatin such as porcine or bovine as well as analogs and homologs thereof of these peptides and proteins. Other suitable larger polypeptides include those disclosed by Pierschbacher et al.,  
10           US 4,589,881 (>30 residues); Bittle et al., US 4,544,500 (20-30 residues); and Dimarchi et al., EP 0 204 480 (>34 residues).

          Other type of compounds useful in the present invention include analogs or homologs of LHRH which display potent LH releasing activity or inhibit the activity of LHRH; analogs or homologs of HP5 which possesses hematopoietic activity; analogs or  
15           homologs of endothelin which possess hypotensive activity; analogs or homologs of enkephalin which have antinociceptive activity; analogs or homologs of cholecystokinin; analogs or homologs of cyclosporin A which have immunosuppressive activity; analogs or homologs of atrial natriuretic factor; peptidergic antineoplastic agents; analogs or homologs of gastrin releasing peptide; analogs or homologs of somatostatin; gastrin antagonists;  
20           bradykinin antagonists; neurotensin antagonists; bombesin antagonists; oxytocin agonists and antagonists; vasopressin agonists and antagonists; hirudin analogs and homologs; analogs and homologs of the cytoprotective peptide-cyclolinopeptide; alpha MSH analogs; analogs, and homologs of MSH releasing factor (Pro-Leu-Gly-NH<sub>2</sub>); peptides which inhibit collagenase; peptides which inhibit elastase, peptides which inhibit renin; peptides  
25           which inhibit HIV protease; peptides which inhibit angiotensin converting enzyme; peptides which inhibit chymases and tryptases and peptides which inhibit blood coagulation enzymes.

          Other suitable drugs include non-peptide therapeutic agents such as antibiotics,  
30           antimicrobial agents, antineoplastic agents, cardiovascular and renal agents, anti-inflammatory, immunosuppressive and immunostimulatory agents and CNS agents.

          Preferably, the drug is a peptide such as a fibrinogen receptor antagonist peptide (an RGD peptide), GHRP (His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), a vasopressin, a calcitonin  
35           or an insulin, more preferably the fibrinogen receptor antagonist peptides cyclo(S,S)-Na-acetyl-Cys-(Na-methyl)Arg-Gly-Asp-Pen-NH<sub>2</sub> or cyclo(S,S)-(2-mercapto)benzoyl-(Na-methyl)Arg-Gly-Asp-(2-mercapto)phenylamide or GHRP.

In a preferred aspect, the present invention provides compositions in the form of microemulsions comprising a peptide which may be orally administered and which will retain biological activity, thereby overcoming the disadvantages of earlier formulations in which the bioavailability of the peptide has been less than satisfactory. In particular, the present invention provides compositions which by their nature permit the preparation and administration of a peptide in sufficiently high concentration to allow not only convenient oral administration but also adequate bioavailability of the peptide.

For a water-soluble drug, the degree of incorporation into (w/o) compositions of the present invention is limited only by its solubility in the hydrophilic phase. By a person skilled in the art, isotonic aqueous phase in the physiological pH range (3 - 8) may be used to aid drug dissolution by the proper modification of the fatty acid/fatty acid salt ratio, without compromising the integrity of the active ingredient and stability of the composition.

The aqueous hydrophilic phase suitably comprises water or an isotonic saline solution and may also include a pharmaceutically acceptable solvent which is non-miscible with the selected lipophilic phase.

It will be readily appreciated by the skilled man that not all blends of an oil, low and high HLB surfactants and hydrophilic phase will yield stable, self-emulsifying microemulsions within the scope of the present invention. Appropriate ratios may, however, be readily determined by the skilled man with the aid of a phase diagram. For the purposes of illustration, the preferred system of a fatty acid salt, a free fatty acid (first low HLB surfactant), an oil, a further, second, low HLB surfactant, and an aqueous solution will be considered. Although this system comprises five components, a pseudo-ternary phase diagram may be constructed by reducing the number of variables to three, by holding two pairs (free fatty acid/fatty acid salt and oil/second low HLB surfactant) each in a fixed ratio. Each of the three variables may then be represented by one side of the triangle. Thus, in figure 1, (1) represents the mixture of oil and second low HLB surfactant, at a fixed ratio X, (2) the hydrophilic (aqueous) phase and (3) the free fatty acid and fatty acid salt at a fixed ratio Y. By way of example, the point "A" represents a microemulsion of 40% oil plus second low HLB surfactant, 10% aqueous phase and 50% free fatty acid plus fatty salt. It will be appreciated by the skilled man that if either the second low HLB surfactant or the free fatty acid is omitted, then the variables (1) or (3) will no longer need to be a fixed ratio and corresponding phase diagrams may be constructed.

The regions of the phase diagram in which microemulsions according to the present invention exist may be determined by titrating a mixture of the oil and second low HLB

surfactant (in a fixed ratio) against the free fatty acid plus fatty acid salt (in a fixed ratio) and the hydrophilic phase, noting points of phase separation, turbidity and transparency. Clear, transparent compositions are indicative of the formation of a stable microemulsion. These compositions may then be plotted on the phase diagram, to generate a microemulsion field, the boundary of which represents the transition from clear, transparent compositions (microemulsions) to turbid compositions, as shown in figure 1.

In addition to the standard method of phase diagrams as illustrated above, phase diagrams were also constructed using the total surfactant present in the system as one component; the oil as a second component; and the hydrophilic phase as third component. A representation of this phase diagram is illustrated as Figure 7 corresponding to Example 6 herein.

Once stable transparent systems are obtained, simple tests, such as dye solubilization, dispersibility in water and conductivity measurements may be used to determine whether the microemulsion is an (o/w)- or a (w/o)-type. A water-soluble dye will disperse in an (o/w) microemulsion while it will remain in its original form in a (w/o) microemulsion. Likewise, (o/w) microemulsions are generally dispersible in water whereas (w/o) microemulsions are generally not. In addition, (o/w) microemulsions conduct electricity whereas (w/o) do not. The isotropic nature of the system may be confirmed by examination thereof under polarized light. The microemulsions are isotropic and therefore non-birefringent when examined under polarized light.

Microemulsions within the scope of the present invention are those falling within the microemulsion existence field of the pseudo-ternary phase diagrams herein.

Accordingly, the present invention provides compositions which form stable, self-emulsifying (w/o) microemulsions as hereinbefore defined in which the relative proportions of the various components lie within the microemulsion existence field of a pseudo-ternary phase diagram such as figure 1.

By this process of constructing a representative range of phase diagrams, for different ratios X and Y, it is possible to determine appropriate quantities of the various components which will lead to stable, self-emulsifying microemulsions falling within the present invention.

Suitably, the oil comprises from about 5 to 95 , preferably from about 10 to 80% (w/w) of the microemulsion.



Suitably, the low HLB surfactant comprises from about 15 to 85, preferably from about 20 to 70% (w/w) of the microemulsion.

5        Suitably, the high HLB surfactant comprises from about 5 to about 75%, preferably about 5 to about 50%, more preferably from about 7.5 to about 30% (w/w) of the microemulsion.

10        Suitably the hydrophilic phase comprises from just greater than 0 to about 40%, preferably from about 0.1 to 20%, more preferably from about 0.1 to 10% and most preferably from about 1 to 5% (w/w) of the microemulsion.

15        It will be readily appreciated by the skilled man that, in general, if it is desired to accommodate a larger amount of hydrophilic phase, this will have to be matched by an increase in the relative amount of high HLB surfactant(s), at the expense of lipophilic components.

20        In preferred microemulsions, the oil plus second low HLB surfactant together comprise from about 8 to about 95%, preferably about 10 to about 90%, more preferably about 40 to about 90%, most preferably about 60 to about 90% (w/w) of the microemulsion. The oil and the second low HLB surfactant may be combined and mixed at various ratios. Useful (w/o) microemulsions which have relatively low viscosity throughout the whole of the microemulsion field may be obtained when the ratio of oil to second low HLB surfactant is in the range of about 5:1 to about 1.5:1, preferably about 4:1 to about 2:1. It is found that as the ratio of oil to second low HLB surfactant is increased towards 5:1, the microemulsion field tends to shrink towards the apex of the phase diagram formed by the sides representing (1) and (3).

30        In preferred microemulsions, the free fatty acid and the fatty acid salt are preferably present in the range of about 5 to about 75%, more preferably about 5 to about 50%, most preferably from about 7.5 to about 30% (w/w) of the microemulsion. The free fatty acid and the fatty acid salt may be combined and mixed at various ratios, for instance in the range of from about 10:1 to 1:1, more preferably in the range of from about 4:1 to 1:1 (w/w).

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The microemulsions of the present invention are substantially non-opaque, that is they are transparent or opalescent when viewed by optical microscopic means. In their undisturbed state, they are optically isotropic (non-birefringent) when examined under

polarized light. They exhibit excellent stability at low and ambient temperatures, without phase separation, clouding or precipitation, even over prolonged periods of time. The formulations may be stored in a stable form at various temperatures, such as at 4°C, room temperature, 37°C and at 50°C, preferably at 4°C or room temperatures. On dilution with  
5 excess aqueous phase, the microemulsions of the present invention tend to invert to (o/w) emulsions.

Preferably, the diameter of droplets or particles of the microemulsions of the present invention, measured, for instance, as the number-average diameter by laser light  
10 scattering techniques, is less than 150 nm, more preferably less than 100 nm, yet more preferably less than 50 nm and most preferably in the range 5 to 35 nm.

The various phases may optionally contain further ingredients, such as, but not limited to:

- 15 i) lipids, such as phospholipids which may be anionic, cationic or zwitterionic, in particular lecithin's, such as soya bean lecithins, egg lecithin or egg phosphatide, cholesterol or long-chain fatty acids such as oleic acid;
- ii) antioxidants such as n-propyl gallate, butylated hydroxyanisole (BHA) and mixed isomers thereof, d- $\alpha$ -tocopherol and mixed isomers thereof, ascorbic acid,  
20 propylparaben, methylparaben and citric acid (monohydrate), for instance in amounts less than 3, preferably less than 1% (w/w);
- iii) bile salts and the alkali metal salts thereof, such as sodium taurocholate;
- iv) stabilizers, such as hydroxypropyl cellulose, for instance in amounts less than 3, preferably less than 1% (w/w);
- 25 v) antimicrobials, such as benzoic acid (sodium salt);
- vi) other anionic surfactants such as dioctylsuccinate, di-octylsodium sulfosuccinate or sodium lauryl sulfate; and
- vii) protease inhibitors such as aprotinin.

30 The present invention includes not only microemulsions which are liquids or gels at room temperature (about 23°C) but also microemulsions which, while liquid at the body temperature of the animal being treated, are solid at room temperature. Such solid microemulsions may be readily prepared by using a high melting oil and, optionally, a high melting low HLB surfactant. Suitably, such oils or low HLB surfactants will have a  
35 melting point above room temperature, preferably above 30°C and examples thereof are well known in the art. Suitable high melting oils include hydrogenated coconut oil and palm oil and blends thereof, such as the HYDROKOTE oils available from Karlshamns Lipid Specialties, hydrogenated peanut oil and various hydrogenated vegetable oils. Also

suitable are mixtures of triesters and diesters of propylene glycerol and lauric acid, such as the product WITEPSOL H-15, available from Huls of America and which contains a 9:1 mixture of tri- and di-esters. Suitable high melting low HLB surfactants include sunflower oil monoglycerides such as the products MYVEROL 18-92 and 18-99.

5

The present invention provides for microemulsions which, upon addition of aqueous fluid, convert to both O/W emulsions and microemulsions. In systems that convert to O/W microemulsions the aqueous phase is preferably a 10-95%, preferably a 20-70%, more preferably a 20-50% by weight a solution of such compounds as sorbitol, polyethylene glycol (PEG), mannitol, propylene glycol, mono- and di-saccharides and mixtures thereof.

10

The microemulsions of the present invention form spontaneously or substantially spontaneously when their components are brought into contact, that is without the application of substantial energy supply, for instance in the absence of high shear energy such as imparted by homogenization and/or microfluidization or other mechanical agitation. Accordingly, the microemulsions may be readily prepared at room temperature by the simple process of admixing appropriate quantities, with gentle hand mixing or stirring, if necessary, to ensure thorough mixing. Preferably, the drug is dissolved in the hydrophilic phase, either directly or by dilution of a stock solution thereof and this may then be added to a pre-mixed combination of the oil and, if being used, the second low HLB surfactant with mixing, followed by the fatty acid salt and, if being used, the free fatty acid and non-ionic high HLB surfactant or vice versa. Alternatively, a drug-free microemulsion may be initially prepared by admixing the oil and surfactants and drug-free hydrophilic phase; to which may then be added further hydrophilic phase in which the drug is dissolved.

Microemulsions which are solid at room temperature may be prepared by using higher temperatures, such that the various components are all liquids, for instance between 40 and 60°C, to facilitate mixing. Such microemulsions may then be allowed to cool down to room temperature, during which solidification occurs.

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Microemulsions of the present invention may be pharmaceutical compositions comprising a therapeutic agent and which may be given to animals, including man.

Accordingly, in a further aspect, the present invention provides a method of treatment which comprises administering an effective amount of a pharmaceutical composition as hereinbefore defined to a patient in need thereof.

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It will be appreciated by the skilled man that the amount of drug required for therapeutic effect will vary with the drug chosen, the nature and severity of the condition

and the animal undergoing treatment and is ultimately at the discretion of the physician. Furthermore, the optimal quantity and spacing of individual dosages of a drug will be determined by the nature and extent of the condition being treated, the form, route and site of administration, the particular patient being treated and that such optima can be  
5 determined by conventional techniques. It will also be appreciated that the optimal course of treatment, that is, the number of doses given, may be readily ascertained using conventional course of treatment determination tests.

In a further aspect, the present invention provides for the use of an oil, a surfactant  
10 system comprising a mixture of high and low HLB surfactants in which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant, a therapeutic agent and a hydrophilic phase, as hereinbefore defined, in the manufacture of a medicament.

15 Pharmaceutical compositions of the present invention may be administered parenterally, enterally or via a mucous membrane, for instance, by injection or by oral, topical, rectal, colonic or intra-vaginal administration. Accordingly the compositions will be presented in forms suitable for such. Thus for instance, pharmaceutical compositions intended for oral administration may be presented in soft gelatin capsules while the  
20 viscosity characteristics of some of the pharmaceutical compositions make them suitable for direct topical application. Solid formulations are preferred for colonic and rectal administration.

The microemulsion compositions of the present invention without a drug are novel  
25 and useful as precursors to drug-containing microemulsions. Accordingly, in a further aspect, the present invention provides a composition comprising an oil; a surfactant system comprising a mixture of high and low HLB surfactants in which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant and an aqueous hydrophilic phase which components on admixing form a stable, self-  
30 emulsifying, water-in-oil (w/o) microemulsion.

The invention will now be illustrated by, but not limited to, the following descriptions (drug-free compositions) and examples (drug-containing compositions) and biological examples, with reference to the afore mentioned figures.

35

## DESCRIPTIONS

### Description 1 - Phase Diagrams for Representative Compositions

Pseudo-ternary phase diagrams were constructed for the following representative systems, with saline as the aqueous phase for all but description 3 which used de-ionized water:

5

Descrip- -tion	Oil/2nd low HLB surfactant	Ratio	Free fatty acid/fatty acid salt	Ratio	Fig.
1	Captex 8000/ Capmul C8	2:1	Caprylic acid/ Na caprylate	3:1	2
2	Captex 8000/ Capmul C8	2:1	Caprylic acid/ Na caprylate	3:1	3
3	Captex 355/ Capmul MCM	3:1	Caprylic acid/ Na caprylate	3:1	4
4	Captex 355/ Capmul MCM	3:1	Capric acid/ Na caprate	3:1	5
5	Captex 355/ Capmul MCM	3:1	Caprylic acid/ Na caprate	3:1	6
6	Captex 200/ Imwitor 308	4.5:1	Capric acid/ Na caprate	2.5:1	7

By way of example, a pseudo-ternary phase diagram was constructed for the system comprising CAPTEX 8000 and CAPMUL C8 (ratio 3:1), caprylic acid and sodium caprylate (ratio 2:1) and aqueous phase (saline or deionized water). The region of the phase diagram in which microemulsions were formed was determined by titrating a mixture of CAPTEX 8000 and CAPMUL C8 against a solution of caprylic acid and sodium caprylate and saline, noting points of phase separation, turbidity and transparency.

The resultant phase diagram is shown as figure 2. A wide range of clear, transparent, liquid (w/o) microemulsions was available. These were stable at room temperature and 37°C. On dilution with excess aqueous phase, inversion to a turbid (o/w) emulsion was observed.

In a similar manner, phase diagrams were constructed for the other systems given in the table and these are shown as figs. 3 to 6. A similar range of microemulsion fields was obtained. The microemulsions were found to have relatively low viscosity throughout the field. In addition, it was noted that when the aqueous phase was changed from saline

to deionized water (description 3), higher levels of aqueous phase could then be accommodated.

### EXAMPLES

- 5        Examples 1-4 describe w/o microemulsions comprising Captex 8000 and Capmul C8 (ratio 3:1); caprylic acid and sodium caprylate (ratio 3:1) and an aqueous phase and either GHRP (His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>) or the model compound calcein (5(6)-carboxyfluorescein MW=623). The relative proportions are given in the the following table:

10

Example	Drug	Drug conc. mg/g form.	CAPTEX 8000 & CAPMUL C8, %(w/w)	Na caprylate & caprylic acid %(w/w)	aqueous phase %(w/w)
1	GHRP	1.4	65	30	5 <sup>a</sup>
2	GHRP	1.4	10	85	5 <sup>a</sup>
3	calcein	1.6	65	30	5 <sup>b</sup>
4	calcein	1.6	10	85	5 <sup>b</sup>

Footnotes to table:

<sup>a</sup> aq. = isotonic soln containing acetic acid and sodium chloride at pH 5.0;

<sup>b</sup> aq = isotonic Tris, pH 7.4.

15

- 20        These microemulsions were formulated by initially preparing the drug-containing hydrophilic phase, either by dissolving the appropriate amount of drug in the appropriate amount of the aqueous phase or, more preferably, using a stock solution which was then further diluted if so required, and with vortex stirring if necessary to obtain complete
- 25        dissolution. The hydrophilic phase containing the drug was then added to the appropriate amounts (by weight) of a mixture of the oil and the second low HLB surfactant, to which was then added a solution of fatty acid salt in free fatty acid, with gentle stirring (magnetic hot plate stirrer). Alternatively, the hydrophilic phase containing the drug was added to the solution of fatty acid salt in free fatty acid. This was mixed completely and then added to
- the oil plus second low HLB surfactant mixture. If necessary, the drug-containing microemulsion was then diluted with the corresponding drug-free microemulsion to adjust the concentration of the drug.

Example 5 (Solid Microemulsion)

A w/o microemulsion incorporating 3.2 mg of Calcein per g of formulation which is solid at room temperature but liquid at 37°C was prepared having the following composition (% w/w): Witepsol H-15 (42.6%), Imwitor 308 (23.8%), Tween 80 (12.4%), Capric Acid (11.4%), Sodium Caprylate (4.8%) and 100 mM solution of Calcein in isotonic 10 mM Tris pH 7.4 (5.0%).

Example 6 (Convertible Microemulsion)

A w/o microemulsion was prepared using the following components (% w/w): Captex 200 (44.5%), Imwitor 308 (9.8%), Tween 80 (19.6%), Capric Acid (10.7%), Sodium Caprate (4.4%), aqueous phase containing a mixture of propylene glycol (4.9%), 100 mM calcein solution (4.9%) and 5N sodium hydroxide (1.2%).

The w/o microemulsion of Example 6 upon a 20-fold dilution with deionized water was converted into an O/W clear microemulsion (final pH of the aqueous phase: 6.9) with an effective particle diameter of 73 nm and polydispersity of 0.364 as determined by laser light scattering.

Example 7 (Solid Microemulsion)

A w/o microemulsion incorporating Calcein in a formulation which is solid at room temperature but liquid at 37°C was prepared having the following composition (% w/w): Witepsol H-15 (42.75%), Imwitor 308 (23.75%), Tween 80 (12.35%), Lauric Acid (11.4%), Sodium Laurate (4.75%) and 100 mM solution of Calcein in isotonic 10 mM Tris pH 7.4 (5.0%).

**METHODS OF TREATMENT**Oral Bioavailability of Calcein

Using a standard unconscious rat model (Walker et al., Life Sciences, 47, 29-36, 1990, see method description under in vivo testing of GHRP-containing microemulsion), the intraduodenal bioavailability of the model compound calcein (5(6)-carboxyfluorescein, MW=623) when dosed as the microemulsion of examples 3 and 4 was assessed and compared with that obtained when the same compound was dosed by the same route but as a solution in isotonic Tris buffer. The levels of the compound in the plasma samples were determined using fluorescence spectroscopy. After i.d. dosing at 2.5 µmol/kg (1.0 ml/kg microemulsion), the bioavailability was 36.3±4.2 (n=5) and 29.9±6.1 (n=5) for the microemulsions of examples 3 and 4 respectively. In comparison, the bioavailability of the same compound administered as an isotonic Tris buffer was only 1.3±0.5% (n=5).

The w/o microemulsion of Example 5 dosed i.d. at calcein dose of 5.0  $\mu\text{mol/kg}$  (1.0 ml/kg microemulsion) produced a bioavailability of  $19.1 \pm 2.7$  (n=5).

- 5        The w/o microemulsion of Example 6 dosed i.d. at calcein dose of 5.0  $\mu\text{mol/kg}$  (1.0 ml/kg microemulsion) produced a bioavailability of  $25.1 \pm 4.6$  (n=5).

The w/o microemulsion of Example 7 dosed i.d. at calcein dose of 5.0  $\mu\text{mol/kg}$  (1.0 ml/kg microemulsion) produced a bioavailability of  $13.8 \pm 2.56$  (n=5).

10

#### Oral Dosing in Rats/GI Irritation Assessment

- One aspect of the present invention are the formulations of w/o self-emulsifying microemulsions with or without peptide which produce little, if any, damage along the GI tract upon oral administration. Formulations of the present invention will be given orally by gavage at a volume not exceeding 10 ml/kg (preferably at three rats per formulation). After 24 hrs the animals are exsanguinated and upon abdominal incisions the gastric and duodenal mucosa are examined both by naked eye and under the microscope (Nikon model SMZ-10 binocular microscope). The mucosal surface of both the stomach and duodenum of the animals that receive microemulsions are examined to see if they are free of any lesions at naked eye.
- 15
- 20

#### Oral Bioavailability of an RGD Peptide in Rats:

- In the procedure described below the microemulsions formulated as hereinbefore and containing a peptide (preferably 3 mg per g of microemulsion) may be tested in the following manner for oral bioavailability.
- 25

##### a) Intravenous (iv) administration of peptide in saline

- Fasted rats are given an intraperitoneal (i.p.) injection and surgically fitted with femoral artery catheters. Rats are allowed to recover from the surgery for 1 day. Catheterized rats are fasted for 18 hr prior to the experiment. Each rat receives 3 mg of peptide by lateral tail-vein administration from a solution prepared as follows:
- 30
- 10.84 mg peptide q.s. to 8 ml with 0.9% saline solution. Blood samples of 0.5 ml aliquots are collected at 0, 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 minutes. The 0 min. sample is taken 15 min. prior to administration of the dose. Plasma is removed from the whole blood by centrifugation at 16000 Xg for 5 min., and then plasma is stored at -20°C in 250 $\mu\text{l}$  aliquots per sample. The blood pellet is reconstituted with heparinized saline and returned to the appropriate rat via catheter. After the experiment, rats were euthanized with iv administration of pentobarbital.
- 35



b) Intraduodenal (i.d.) administration of peptide in microemulsion

Fasted rats are given an i.p. injection of anesthesia cocktail and surgically fitted with jugular and duodenal catheters. Rats are allowed to recover from the surgery for 4-5 days. Catherized rats are fasted 18-20 hrs. prior to the experiment. Each rat receives 10mg of peptide in either microemulsion or saline solution. Blood samples of 0.5ml aliquots are collected via jugular catheter in heparinized eppendorf tubes at 0, 10, 30, 60, 120, 180, 240 and 1440 minutes. The 0 min sample is taken 15 min prior to administration of the dose by duodenal catheter. Plasma is collected for analysis and the blood returned to rats as described in the i.v. administration (part a) above. After 1440 min, rats are euthanized by iv administration of pentobarbital, exsanguinated and the GI tract removed for gross observation.

c) Analysis of peptide plasma concentration

Standards were placed before and after the sample for HPLC analysis. A 50µl aliquot for 0-200 ng peptide, 25µl aliquot for 1000-2000 ng peptide, 15µl aliquot for 10,000 ng peptide and a 50µl aliquot of each sample is analyzed by post-column fluorescence detection. Fluorescence chromatography data is collected and integrated using a Nelson Chromatography Data System. The peak area ratio (Y) and peptide standard concentration (X) are used to determine the slope of a line which is forced through the origin from the equation:  $\text{slope} = (\text{sum of } X \cdot Y) / (\text{Sum of } X^2)$ . The slope represents the relationship between peak area ratio and peptide plasma concentration for the samples.

d) Calculation of Bioavailability

First, the area under the plasma concentration curve (AUC) from 0 to 240 minutes is determined for each rat. For id administration, percentage bioavailability is determined for each animal by the following equation with the average AUC from iv administration:  $[(\text{AUC}_{\text{id}}/\text{AUC}_{\text{iv}}) \cdot (\text{dose iv}/\text{dose id})] \cdot [100]$ .

The oral bioavailability data for the RGD peptide in rats after intraduodenal administration of a microemulsion containing the above formulations incorporating a fibrinogen receptor antagonist of a peptide dose may then be obtained in the above noted manner.

When applicable, the formulations of the present invention are tested for in vivo activity. As one of the active ingredients utilized herein is a fibrinogen receptor antagonist a platelet aggregation assay is employed to determine pharmacological activity of the peptide from microemulsions. These studies are carried out as shown below.

Oral Dosing in Dogs/Platelet Aggregation Assay :

Dogs which may be used in this assay are, for instance, male Mongrels (i.e. from mixed breeds). The dog(s) are fasted overnight the day before the experiment. The cephalic vein of choice is prepared for the indwelling catheter in the following way: the area is first shaved and cleaned with a gauze soaked in 70% alcohol. An indwelling catheter is placed in the cephalic vein and attached to a luer lock adapter filled with 3.8% sodium citrate. The catheter is securely taped down. When a blood sample is withdrawn, a 0.3 ml of blood is withdrawn into a separate 1 cc syringe before the actual sample so that dilution of the blood sample from the sodium citrate contained in the luer lock adapter is avoided. Then 2.7 ml of blood are drawn in a 3 cc syringe and placed in a Venoject vacuum tube containing 0.3 ml of 3.8% sodium citrate and labelled with the appropriate time point. The tube containing the blood sample in 3.8% sodium citrate is gently inverted few times to mix components and then 1 ml is withdrawn for the whole blood aggregation assay. The rest of the blood sample is transferred to an eppendroff tube and upon centrifugation the supernatant plasma is removed and transferred to a new tube which is then frozen for subsequent HPLC analysis to determine peptide content.

Just after the zero time point blood sample is withdrawn, an appropriate dose of microemulsion with or without peptide is administered orally to the dog using a size 12 gelatin capsule.

The blood samples are then assayed for platelet aggregation inhibition using the Chromo-Log whole blood aggregometer. The instrument is warmed to 37°C before samples are run and the probe is cleaned with distilled water and a soft brush. The probe is attached to the aggregometer and placed in a cuvette of saline solution and warmed in a side cuvette well in the aggregometer. For the actual assay, 1 ml of the 2.7 ml of blood sample mixed with the 0.3 ml 3.8% sodium citrate contained in the Venoject vacuum tube is added to a cuvette and placed in the aggregometer well. A stir bar is placed in the cuvette and set at 900 rpm. The probe is placed firmly into the test cuvette and the lid is shut. Baselines, zero and calibration are set. Calibration is set equal to  $20 = 5$  ohms. The stirring cuvette is permitted to settle for five minutes at which point 5  $\mu$ l of collagen is added to the whole blood that is being stirred to yield to a 5  $\mu$ g/ml final solution in the cuvette.

The reaction is monitored for two minutes once the slope change reaches the baseline of the collagen addition, calculating the change in ohms per minute using the slope of the two minutes. The change in ohms per minute is calculated as a % of the control. The control value is determined by the average of the -15 and the 0 time points. After each use the probe is removed and cleaned with distilled water and wiped with a soft cloth and brush.

#### Discussion and Conclusion:

A dog is considered a good model to assess the pharmacological effect of one class of peptides of interest herein, the RGD containing fibrinogen receptor antagonists.

- 5 Experiments are conducted as described above, with a peptide dose of 3 mg/kg or microemulsion dose of 0.5 ml/kg. Control experiments where the peptide is given orally in a saline solution are independently carried out earlier and serve as a useful comparison to the effects seen with the microemulsion-formulated peptide.

- 10 As one of the active ingredients utilized herein is a Growth Hormone Releasing Peptide the appropriate assay for in vivo activity is determined as shown below.

#### In Vivo Testing of GHRP-Containing Microemulsion:

- 15 Microemulsions with a composition (w/w) in accordance with the present invention of Examples 1 and 2 are made. Upon preparation they are further stored in a stable form at ambient temperature for approximately 48 hrs before the in vivo evaluation. A control solution of a GHRP peptide, His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>, in saline at 1.5 mg/ml is also prepared.

- 20 Dosing was done by single intraduodenal administration of GHRP at 1.35 mg/kg in male rats in saline solution (control) and in the aforementioned microemulsion using 5 rats in each case. Prior to actual sampling and dosing, each rat was anesthetized with Pentobarbital at 50 mg/kg i.p., diluted with saline to a final volume of 1 ml. The rats stayed anesthetized for the entire experiment. Dosing was achieved in the following way: a small  
25 incision 2-3 cm long was made on the abdominal midline, and then a purse-string suture was placed on the duodenal muscle. A small hole was made in the center of the purse-string suture in which a blunt 23 G stub needle attached to a tuberculin syringe was inserted to deliver the dose. Upon completion of dosing, the purse-string was tied to close the opening. The incision was closed with wound clips. A 0.2 ml blood sample was obtained  
30 via jugular catheter at the following intervals: -15, 0, 5, 10, 15, 30, 45, 60, 90, and 120 minutes. Blood samples were stored on ice and subsequently analyzed for Growth Hormone by an RIA method.

- 35 Analysis of the samples generated from the experiment mentioned above to determine pharmacological activity of GHRP, indicated significant GH levels in the blood from the peptide-containing microemulsion-treated animals whereas with the control saline solution of GHRP the GH levels seen were negligible. The data indicates that the Growth Hormone Releasing Peptide is orally active from the microemulsion formulation of the

- 26 -

present invention. However, blood levels and actual bioavailability have not been correlated to observed pharmacological activity.

5       The amount of active ingredient required for therapeutic systemic administration will, of course, vary with the compound chosen, the nature and severity of the condition, and the mammal, including humans, undergoing treatment, and is ultimately at the discretion of the physician.

10       Ultimately, the present invention also includes a method of treatment which comprises administering an effective amount of a pharmaceutical composition as defined herein to a patient in need thereof. Preferably, the therapeutic agent is selected from fibrinogen receptor antagonist peptide, Growth Hormone Releasing Peptide, vasopressin, elcatonin, calcitonin, calcitonin-gene related peptide, porcine somatostatin, or insulin. The disease states and uses of each of the aforementioned therapeutic agents is well  
15       known to those skilled in the art and for a number of the agents already cross referenced to their respective patents. For instance, use as platelet aggregation inhibitors, growth promoters, for osteoporosis, and diabetes.

20       The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.  
25       The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A pharmaceutical composition comprising:
  - (a) an oil;
  - 5 (b) a surfactant system comprising a mixture of high and low HLB surfactants in which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant;
  - (c) an aqueous hydrophilic phase; and
  - (d) a water-soluble biologically active agent;
- 10 which on admixing form a stable, self-emulsifying, water-in-oil (w/o) microemulsion.
2. A composition as claimed in claim 1 in which the oil is a pharmaceutically acceptable fatty acyl triglyceride, a fatty acyl diester of propylene glycol or a mixture thereof.
- 15 3. A composition as claimed in claim 2 in which the oil is an interesterified triglyceride of long and medium chain fatty acyl moieties; a physical blend of long and medium chain fatty acyl moieties; a long chain fatty acyl triglyceride; or a medium chain fatty acyl triglyceride.
- 20 4. A composition as claimed in claim 2 in which the fatty acyl triglyceride or fatty acyl diester comprises medium-chain fatty acyl moieties.
5. A composition as claimed in claim 4 in which the fatty acyl triglyceride or fatty acyl diester comprises caprylic acid optionally admixed with capric acid moieties.
- 25 6. A composition as claimed in claim 1 in which the salt in the medium-chain fatty acid salt is a pharmaceutically acceptable water-soluble alkali metal salt, a pharmaceutically acceptable ammonium salt or a pharmaceutically acceptable quaternary ammonium salt.
- 30 7. A composition as claimed in claim 6 in which the medium-chain fatty acid salt is a salt of caprylic or capric or lauric acid.
8. A composition as claimed in claim 7 in which the medium-chain fatty acid salt is a salt of caprylic or capric acid.
- 35 9. A composition as claimed in claim 1 in which the low HLB surfactants are selected from medium or long-chain fatty acyl monoglycerides, medium or long chain fatty acyl

diglycerides, sorbitan long-chain fatty acyl esters or medium-chain free fatty acids, or mixtures thereof.

10. A composition as claimed in claim 9 in which the fatty acyl mono- and di-glycerides  
5 are formed from caprylic and capric acids.

11. A composition as claimed in claim 9 which comprises from about 50 to 100% caprylic acid and from 0 to 50% capric acid mono- and/or di-glycerides.

10 12. A composition as claimed in claim 9 in which the medium-chain fatty acid is caprylic acid, capric acid, lauric acid or a mixture thereof.

13. A composition as claimed in any one of claims 1 to 9 in which the medium-chain fatty acid salt and a free medium-chain fatty acid are present in a ratio of salt to free acid in  
15 the range of from about 1:1 to 1:10.

14. A composition as claimed in claim 9 in which the low HLB surfactant is a blend of medium-chain fatty acyl monoglycerides, medium-chain fatty acyl diglycerides and medium-chain free fatty acids.

20

15. A composition as claimed in claim 1 which comprises medium-chain fatty acyl components.

16. A composition as claimed in claim 15 in which the medium-chain fatty acyl  
25 components are caprylic and/or capric acids or derived therefrom

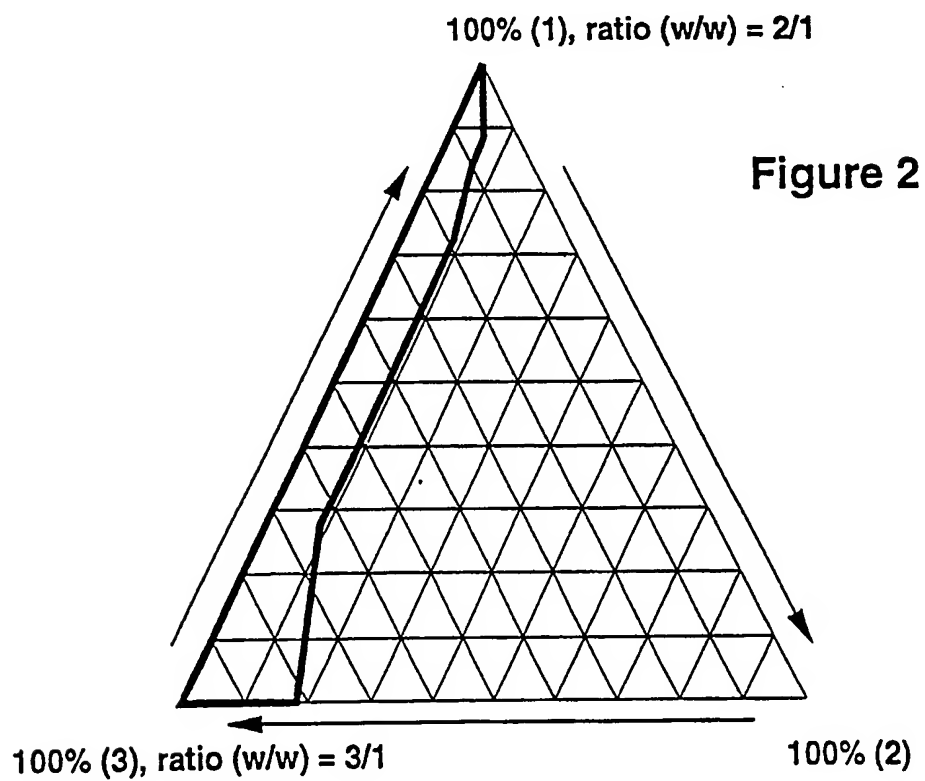
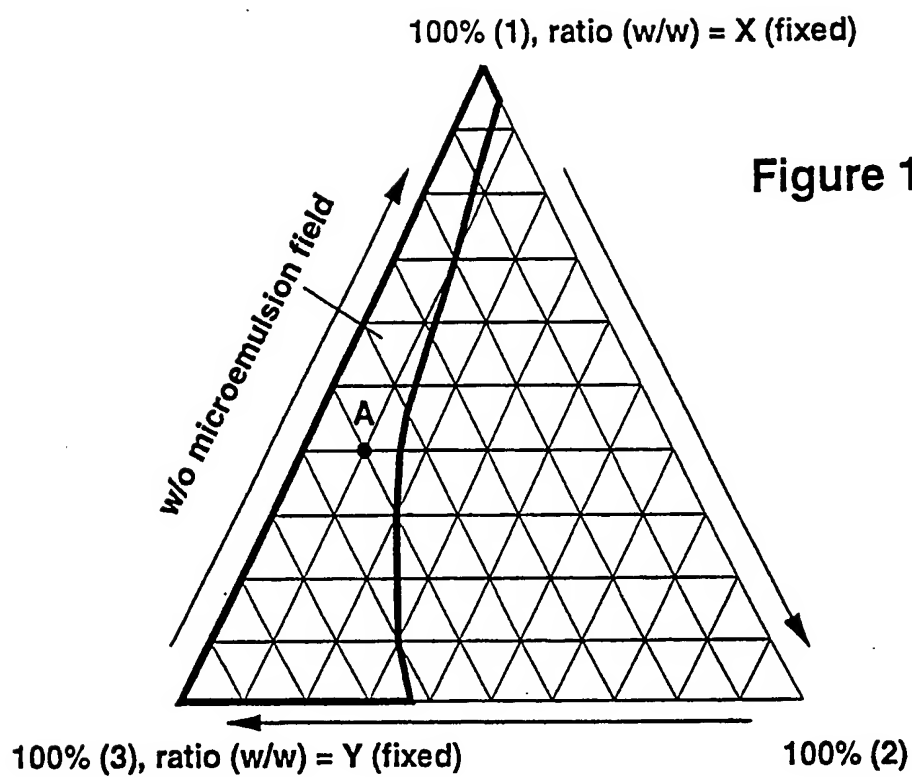
17. A composition as claimed in claim 1 in which the biologically active material is a therapeutic agent which is a peptide.

30 18. A composition as claimed in claim 17 in which the peptide is a fibrinogen receptor antagonist peptide, a Growth Hormone Releasing Peptide, a vasopressin, a calcitonin or an insulin.

19. A composition as claimed in claim 1 which comprising a high melting oil and/or a  
35 high melting low HLB surfactant and which is a solid at room temperature but at liquid at body temperature.

20. A composition as claimed in claim 1 in which the relative proportions of the oil, surfactants and aqueous phase lie within the microemulsion existence field of the pseudo-ternary phase diagrams of Figures 2 to 7.
- 5 21. A composition which comprises an oil; a surfactant system comprising a mixture of high and low HLB surfactants in which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant and an aqueous hydrophilic phase which components on admixing form a stable, self-emulsifying, water-in-oil (w/o) microemulsion.
- 10 22. Use of a pharmaceutical composition as defined in claim 1 in the manufacture of a medicament wherein the thereapeutic agent is selected from fibrinogen receptor antagonist peptide, Growth Hormone Releasing Peptide, vasopressin, calcitonin or insulin.
- 15 23. A process for the production of a pharmaceutical composition which process comprises
- (i) admixing
- (a) an oil;
- (b) a surfactant system comprising a mixture of high and low HLB surfactants in
- 20 which the high HLB surfactant is a medium-chain fatty acid salt optionally admixed with a non-ionic high HLB surfactant; with
- (c) an aqueous hydrophilic phase; and
- (d) a water-soluble biologically active agent; and
- (ii) forming a stable, self-emulsifying, water-in-oil (w/o) microemulsion.

1/4





2/4

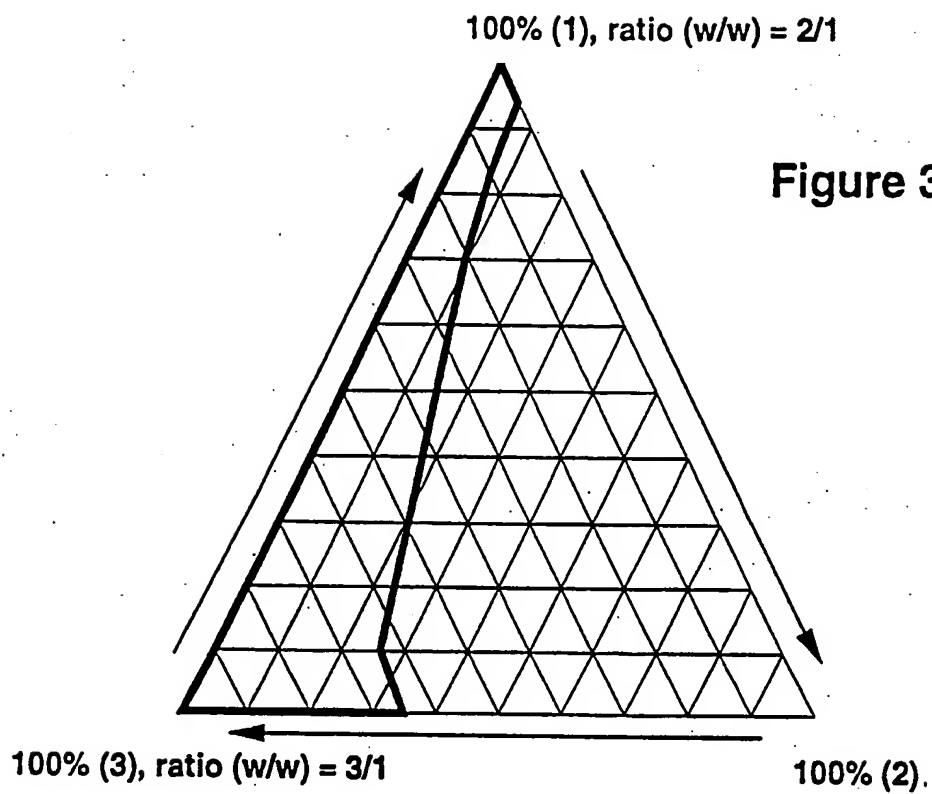


Figure 3

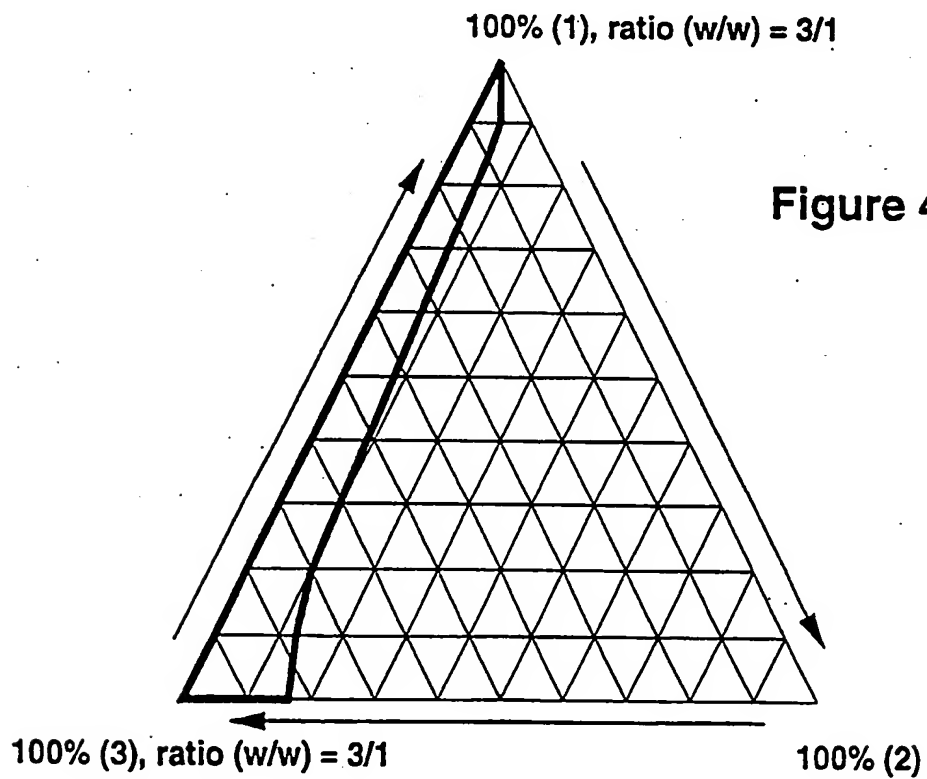
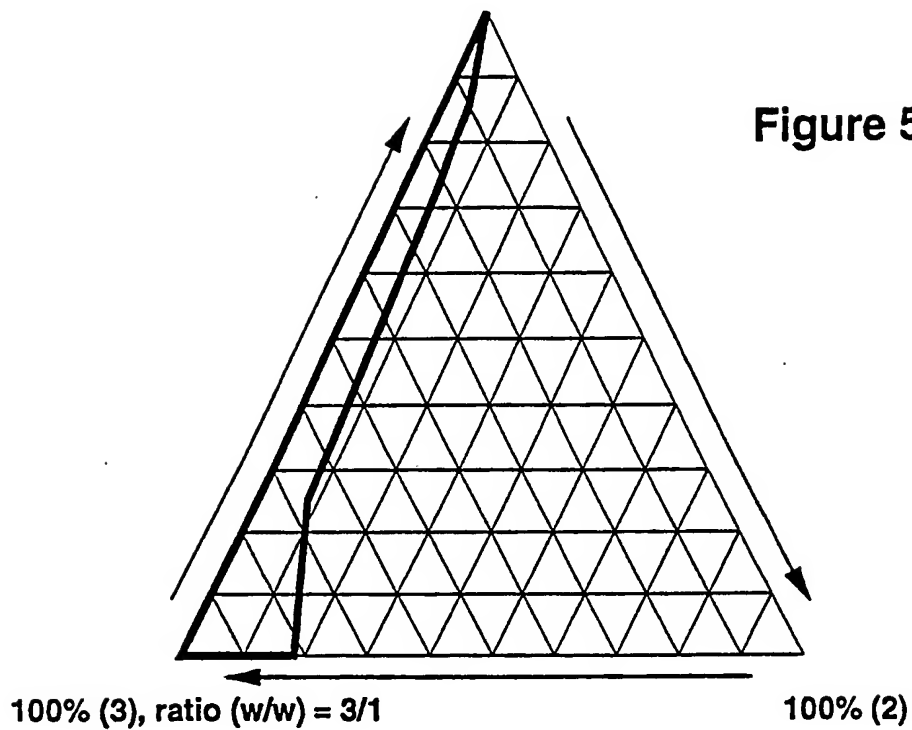


Figure 4

$\frac{3}{4}$

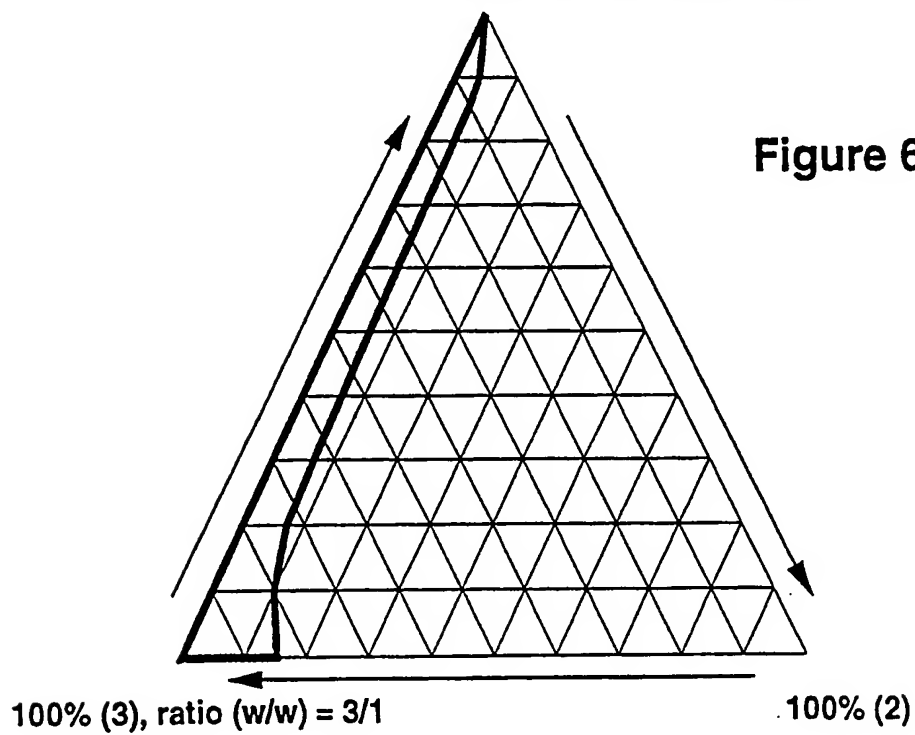
100% (1), ratio (w/w) = 3/1

Figure 5



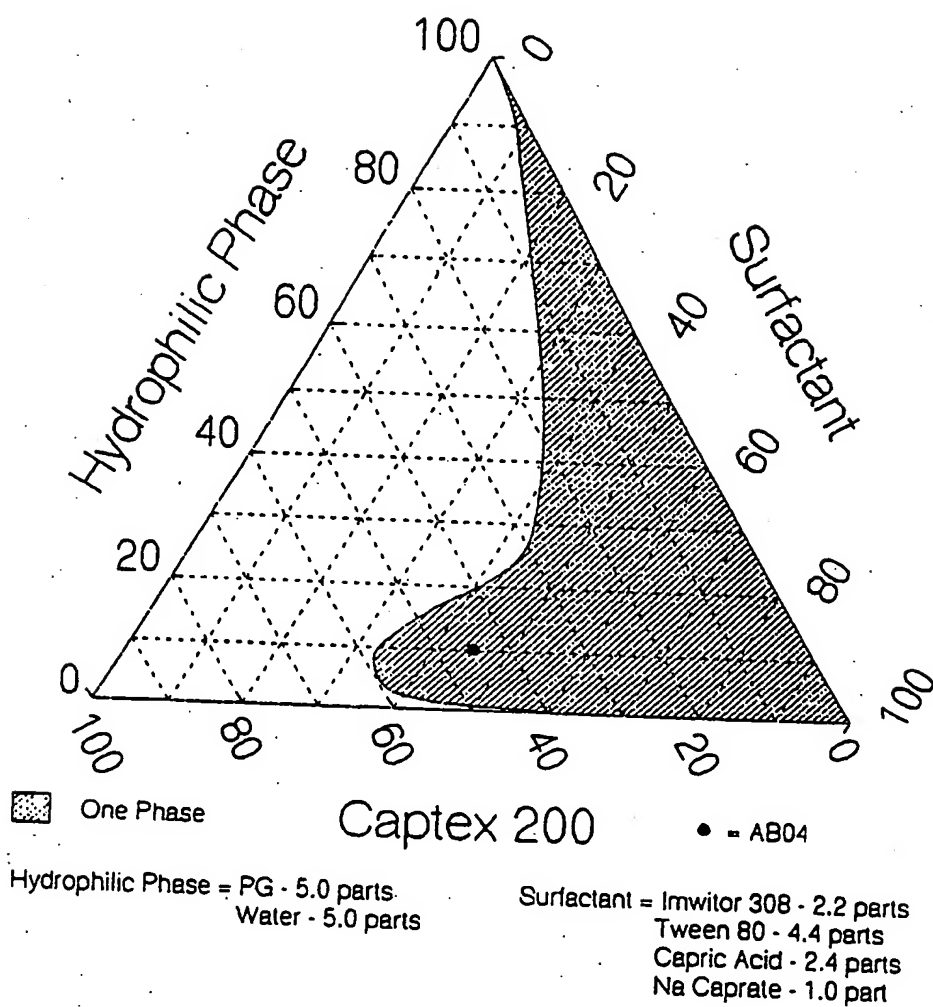
100% (1), ratio (w/w) = 3/1

Figure 6



4/4

Figure 7



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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/09916

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : A61K 37/02, 37/24, 37/26, 37/36, 47/00

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/61, 168, 172, 365; 514/004, 009, 012, 785, 786, 937, 938, 941, 943, 962, 966, 967

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,719,239 (B.W.W. Muller et al) 12 January 1988, see the entire document particularly the abstract and column 6, lines 39-44.	1-23
Y	GB, A, 1,171,125 (C.A. Walton et al) 19 November 1969, see the entire document, particularly, page 1 lines 24-45.	1-23
Y	GB, A, 2,098,865 (J. Franz) 01 December 1982, see the entire document, particularly abstract and page 2, lines 47-58.	1-16, 19-23



Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents:

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\*O\* document referring to an oral disclosure, use, exhibition or other means

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later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

06 JANUARY 1994

Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/09916

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/61, 168, 172, 365; 514/004, 009, 012, 785, 786, 937, 938, 941, 943, 962, 966, 967

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